99D-103

APPLICATION FOR FEDERAL ASSISTANCE

OMB Approval No. 0348-0043

2. DATE SUBMITTED	Application Identifier
3. DATE RECEIVED BY STATE	State Application Identifier
4. DATE RECEIVED BY FEDERAL	Federal Identifier

1. TYPE OF SUBMISSION:
Application: ☐ Construction ☒ Non-Construction
Preapplication: ☐ Construction ☐ Non-Construction

5. APPLICATION INFORMATION

Legal Name: The Regents of the University of California

Address (give city, county, state, and zip code):
University of California, Berkeley
Sponsored Projects Office
336 Sproul Hall, Alameda County
Berkeley, CA 94720-5940

Organizational Unit: Dept. of Biochemistry and Molecular Biology, UCB

Name and telephone number of the person to be contacted on matters involving this application (give area code)

Administrative Contact
Lynn E. Deetz
(510) 643-6113

Technical Contact
Terrance Leighton
(510) 642-1620

6. EMPLOYER IDENTIFICATION NUMBER (EIN): 94-6002123

7. TYPE OF APPLICANT: (enter appropriate letter in box) I

A. State
B. County
C. Municipal
D. Township
E. Interstate
F. Intermunicipal
G. Special District
H. Independent School Dist.
I. State Controlled Institution of Higher Learning
J. Private University
K. Indian Tribe
L. Individual
M. Profit Organization
N. Other (Specify):

8. TYPE OF APPLICATION:
☒ New ☐ Continuation ☐ Revision
If Revision, enter appropriate letter(s) in boxes(es) ☐ ☐
A. Increase Award B. Decrease Award C. Increase Duration
D. Decrease Duration Other (specify):

9. NAME OF FEDERAL AGENCY: CALFED

10. CATALOG OF FEDERAL DOMESTIC
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives

12. AREAS AFFECTED BY PROJECT (cities, counties, states, etc.)
California

13. PROPOSED PROJECT:

Start Date	Ending Date
7/1/99	6/30/01

14. CONGRESSIONAL DISTRICTS OF:
a. Applicant: 9th
b. Project: 9th

15. ESTIMATED FUNDING:

a. Federal	$ 646,645
b. Applicant	$
c. State	$
d. Local	$
e. Other	$
f. Program Income	$
g. TOTAL	$ 646,645

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?
a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE
b. NO. ☐ PROGRAM IS NOT COVERED BY E.O. 12372
☐ OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?
☐ Yes If "Yes," attach an explanation. ☒ No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED

a. Typed Name of Authorized Representative	b. Title	c. Telephone number
Lynn E. Deetz	Senior Research Administrator	(510) 643-6113

d. Signature of Authorized Representative: Lynn E Deetz
e. Date Signed: 4/15/99

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Standard Form 424 (REV 4-88)
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I-018498

I-018498

PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives

Applicant Name: Professor Terrance Leighton

Mailing Address: BMB, 401 Barker Hall, University of California, Berkeley, California 94720-3203

Telephone: 510 642-1620

Fax: 510 643-5035

Email: Leighton@Socrates.Berkeley.Edu

Amount of funding requested: $480,000 for 2 years

Indicate the Topic for which you are applying (check only one box).

	Fish Passage/Fish Screens		Introduced Species
	Habitat Restoration		Fish Management/Hatchery
	Local Watershed Stewardship		Environmental Education
■	Water Quality		

Does the proposal address a specified Focused Action? X yes _____no

What county or counties is the project located in? **Merced**

Indicate the geographic area of your proposal (check only one box):

	Sacramento River		Mainstem East Side Trib:
	Sacramento Trib:		Suisun Marsh and Bay
■	San Joaquin River		Mainstem North Bay/South Bay:
	San Joaquin Trib:		Landscape (entire Bay-Delta watershed)
	Delta:		Other:

Indicate the primary species which the proposal addresses (check all that apply):

- ■ San Joaquin and East-side Delta tributaries fall-run chinook salmon
- ■ Winter-run chinook salmon
- ■ Spring-run chinook salmon
- ■ Late-fall run chinook salmon
- ■ Fall-run chinook salmon
- ■ Delta smelt
- ■ Longfin smelt
- ■ Splittail
- ■ Steelhead trout
- ■ Green sturgeon
- ■ Striped bass
- ■ Migratory birds
- ■ All chinook species
- Other:
- ■ All anadromous salmonids

Specify the ERP strategic objective and target (s) that the project addresses. Include page numbers from January 1999 version of ERP Volume I and II:
This proposal addresses strategic objectives and targets in the February 1999 version of ERP Volume 1 including High Priority At-risk Species, At-risk Native Species and Declining Native Species (pp. 176-363). This proposal addresses strategic objectives and targets in the February 1999 version of ERP Volume 2 including the San Joaquin River Ecological Management Zone (pp. 385- 404) and West San Joaquin Basin ecological Management Zone (pp. 444-455). This proposal addresses strategic objectives in the Revised Draft Water Quality Plan (January 1999) contained in Section 8. Selenium (pp. 8-1 – 8-15).

Indicate the type of applicant (check only one box):

- State agency
- Federal agency
- Public/Non-profit joint venture
- Non-profit
- Local government/district
- Private party
- ■ University
- Other:

I-018500

Indicate the type of project (check only one box):

	Planning		Implementation
■	Monitoring		Education
	Research		

By signing below, the applicant declares the following:

1.) The truthfulness of all representations in their proposal;

2.) The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and

3.) The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

Terrance Leighton

Printed name of applicant

Signature of applicant

Approved for the University of California at Berkeley
Lynn E. Beetz 4/15/99
Senior Research Administrator
Sponsored Projects Office, 336 Sproul Hall
Berkeley CA 94720-5940
(510) 643-6113 FAX: (510) 642-8236

II. TITLE PAGE

FOCUS AREA : WATER QUALITY

(a) Project Title: Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives

(b) Names of Principal Investigator and Co-Investigators

Terrance Leighton: (510) 642-1620
University of California
401 Barker Hall
Berkeley, CA 94720

Nigel Quinn: (510) 486-7056
Lawrence Berkeley National Laboratory
1 Cyclotron Road, 70A-3317K
Berkeley, CA 94720

Richard Higashi: (530) 752-1450
University of California
Crocker Nuclear Laboratory
Davis, CA 95616

Teresa Fan: (530) 752-1450
University of California
Department of Land, Air and Water Resources
Davis, CA 95616

(c) Type of Organization and Tax Status: University 501(C)(3)

(d) Tax Identification Number: **1946002123**

(e) Technical Contact Person:

Dr. Terrance Leighton
Biochemistry and Molecular Biology, University of California
401 Barker Hall, Berkeley, CA 94720
(510) 642-1620; Leighton@Socrates.Berkeley.Edu

(f) Contractual Contact Person:

Ms. Lynn E. Deetz
Sponsored Projects Office
336 Sproul Hall
University of California
Berkeley CA, 94720-5940
(510) 643-6113: ldeetz@uclink4.berkeley.edu

(g) Participants/Collaborators in Implementation:

UCB	LBNL	UCD
Terrance Leighton	Nigel Quinn	Richard Higashi Teresa Fan

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III. EXECUTIVE SUMMARY

Project Title: Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives

Name of Applicants:

Terrance Leighton	Professor of Microbiology, University of California, Berkeley Leighton@Socrates.Berkeley.Edu, (510) 642-1620,
Nigel Quinn	Staff Geological Scientist, Lawrence Berkeley National Lab. nwquinn@lbl.gov, (510) 486-7056
Richard Higashi	Assistant Research Professor, Crocker Nuclear Laboratory, University of California, Davis rmhigashi@ucdavis.edu, (530) 752-0952
Teresa Fan	Associate Research Professor, Department of Land air and Water Resources, University of California, Davis xfan@ucdavis.edu, (530) 757-3045

Project Description:

The Bay-Delta, the largest estuarine system on the west coast of North and South America, supplies two-thirds of the state's population with drinking water, drains over 40 percent of California's land, and irrigates 200 types of crops growing on 4.5 million acres of farmland. These waterways supply and sustain fisheries, wildlife refuges, and 40,000 acres of wetlands. The estuary ecosystem is immensely productive, supporting a diverse community of plant, animal and aquatic life. More than 750 species of fish, animals, and birds, including half of the waterfowl migrating on the Pacific Flyway, use Bay-Delta wetlands for wintering and habitat. The biodiversity of the Bay-Delta ecosystem is critically dependant on the quality and quantity of water contained within the estuary.

This project addresses a critical knowledge gap in our understanding and management of the Bay-Delta ecosystem: the role of the microbiota, which form the base of the ecosystem food web, in affecting selenium fate and transport. This project proposes the isolation, characterization, analysis and monitoring of microbial communities contained in agricultural drainage and wetland return flows generated within the Grasslands Drainage Basin on the westside of the San Joaquin Valley and in the San Joaquin River. These will be referred to as San Joaquin River Basin (SJRB) microbial communities. The bioaccumulation and biotransformation of selenium by these SJDS microorganisms will be studied in controlled laboratory environments, free flowing aquatic ecosystems and engineered biological treatment systems. The processes controlling the fate and nature of selenium assimilation by microbiota will be elucidated. Advanced environmental measurements methods will be developed to determine directly the distribution and chemical species of selenium present in representative microbiota. Experimental systems will be developed to evaluate the bioavailability of microbially incorporated selenium to higher trophic levels of the food chain. Near real-time monitoring systems will be developed to "fingerprint" seasonal and treatment system changes in microbiota community structure and function. The results from this

project will fill crucial data gaps in our understanding of the role of microbiota in selenium dynamics in the SJDS and in a biological treatment facility located in the Panoche Water District. The project results will also provide more realistic concentration objectives for the San Joaquin River and its major tributaries that will both be more protective of the environment and allow agriculture to make use of the true assimilative capacity of the San Joaquin River. The current selenium objectives are neither seasonal nor site specific and hence are inherently inefficient. The project will complement the current CALFED- sponsored Real-Time Water Quality Management project, the long term goal of which is to expand forecasting to cover both selenium and boron in addition to electrical conductivity.

Proposed scope of work:
We propose to :

1. Characterize microbial community structure, function and dynamics in the CALFED-sponsored selenium biotreatment facility located in the Panoche Water District. The CALFED goal of the following experiments is to improve the efficiency and cost effectiveness of the biotreatment facility by developing an improved understanding of the microbial ecology of each unit process in the treatment train and the biokinetics of the treatment process.
    - 1.1. Assess microbial community structure and seasonal variation by direct isolation of microbiota and subsequent classification using Biolog and 16S rRNA gene sequencing methods.
    - 1.2. Assess the effects of treatment system operating parameter variations on microbial community structure.
    - 1.3. Assess the ability of representative isolates and communities to assimilate and biotransform selenate and selenite into organic, elemental and volatile selenium species.
        - 1.3.1. Advanced environmental measurement methods including GC/MS, XANES and EXAFS will be used to determine selenium fate and chemical species.
    - 1.4. Assess the effects of treatment system operating parameter variation on microbial community assimilation and biotransformation of selenate and selenite into organic, elemental and volatile selenium species.
    - 1.5. Develop near real-time selenium treatment system microbiota monitoring methods by using Biolog profiling of microbial community metabolic signatures.
        - 1.5.1. Correlate variations in Biolog community level physiological profiles (CLPP) with variations in treatment system operating parameters.
        - 1.5.2. Develop a CLPP database documenting normal, abnormal and recovering microbial community signatures typical of potential variations in treatment system operating modes.
2. Characterize microbial community structure, function and dynamics in Mud Slough and the San Joaquin River. Mud Slough is the major conveyance of salt and selenium to the San Joaquin River since the implementation of the Grassland Bypass project in 1996, which diverted agricultural drainage into the San Luis Drain. The selenium objective of 5 ppb is continuously exceeded in Mud Slough downstream from the terminus of the San Luis

Drain. The CALFED goals of the following experiments are designed to formulate a more realistic seasonal, site-specific selenium objective for Mud Slough.

2.1. Assess microbial community structure and seasonal variation by direct isolation of microbiota and subsequent classification using Biolog and 16S rRNA gene sequencing methods.

2.2. Assess the effects of drainage system operating parameter variations on microbial community structure.

2.3. Assess the ability of representative isolates and communities to assimilate and biotransform selenate and selenite into organic, elemental and volatile selenium species.

2.3.1. Advanced environmental measurement methods including GC/MS, XANES and EXAFS will be used to determine selenium fate and chemical species.

2.4. Assess the effects of drainage system operating parameter variation on microbial community assimilation and biotransformation of selenate and selenite into organic, elemental and volatile selenium species.

2.5. Develop near real-time drainage system microbiota monitoring methods by using Biolog profiling of microbial community metabolic signatures at Mud Slough and San Joaquin sites.

2.5.1. Correlate variations in Biolog community level physiological profiles (CLPP) with seasonal fluctuations in the drainage system environment.

2.5.2. Develop a CLPP database documenting microbial community signatures typical of variations in drainage system operating modes.

2.5.3. Correlate CLPP signature data with real-time monitoring project data to develop selenium adaptive management strategies.

2.6. Assess the ecotoxic risks of bioincorporated selenium species by pure compound and biomass foodchain transfer studies with two resident Bay-Delta species, red swamp crayfish (*Procambarus clarkii*) and mosquito fish (*Gambusia*) receptors.

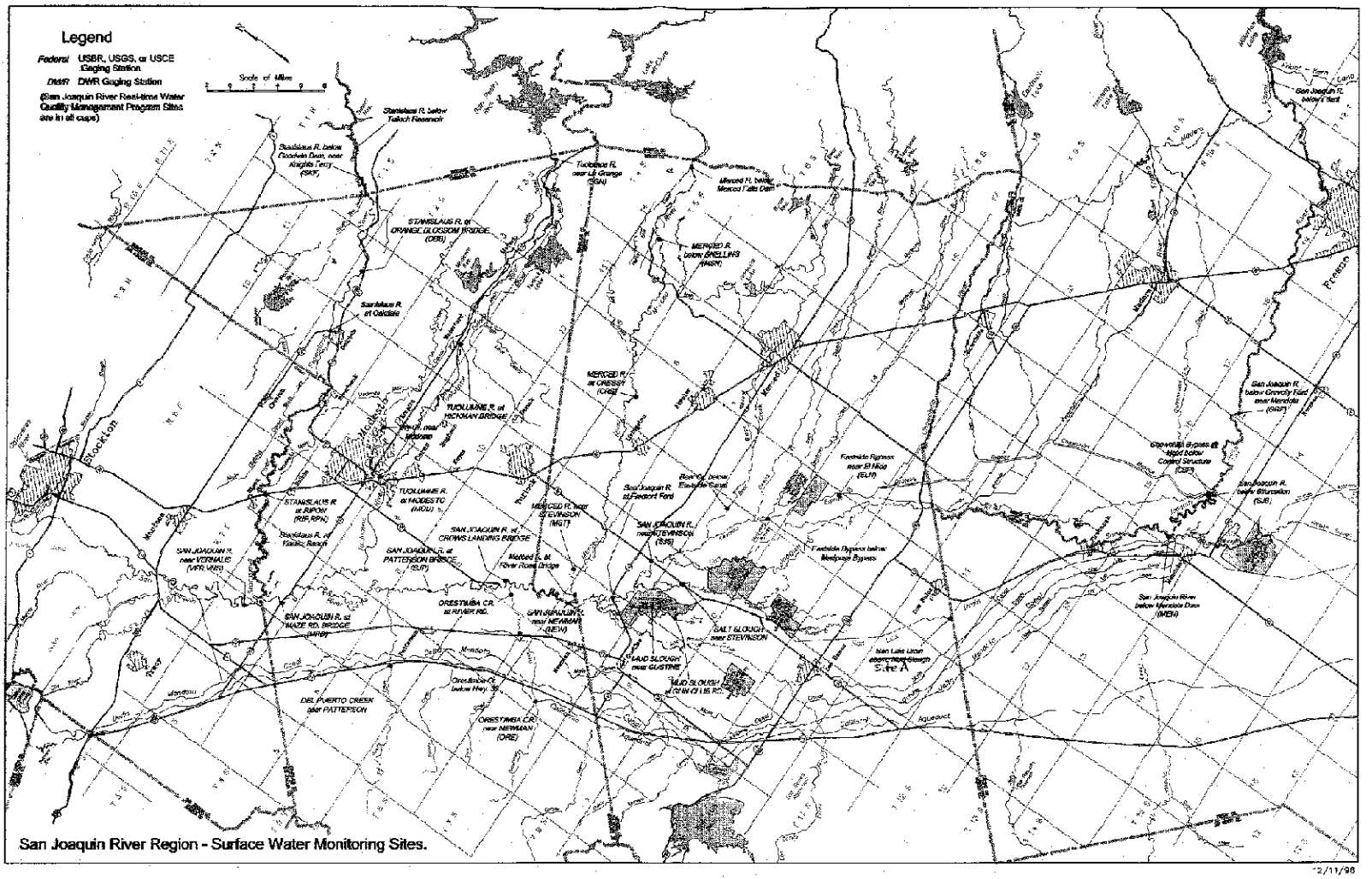
Location:

The study sites are located within Merced County. The project area includes drainage that is conveyed to the San Joaquin River through Mud Slough. The map on the following page shows the San Joaquin River and its major tributaries as well as the Mud Slough and San Joaquin River water quality monitoring sites.

Budget Costs: (2 year duration): Total cost: $480,000

TASK NO.	UCB	LBNL	UCD	TRAVEL / SUPPLIES / EQUIPMENT
Task 1	80,000	30,000	60,000	40,000
Task 2	80,000	50,000	60,000	80,000
TOTALS	160,000	80,000	120,000	120,000

LOCATION AND GEOGRAPHICAL BOUNDARIES OF THE PROJECT



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Applicant qualifications:
The team members include UCB, LBNL, and UCD personnel all of whom have worked in the SJDS for the past five to ten years. The UCB group has specialized in developing tools for the analysis of microbial community structure, function and dynamics in selenium impacted environments. The UCB group has also developed X-ray absorption spectroscopy tools for the *in situ* determination of selenium species and distribution in microbial biomass. The LBNL group has specialized in SJDS selenium fate and transport experiments. LBNL has also developed fate and transport models to support real-time adaptive management of selenium loading. The UCD group has specialized in developing tools for the analysis of algal community structure, function and dynamics in selenium impacted environments. The UCD group has developed GC/MS tools for the determination of selenium species and distribution in algal biomass. The UCD group is recognized for their ability to assess selenium foodchain transfer characteristics and ecotoxic risk.

Key personnel include:

UCB	LBNL	UCD
Terrance Leighton Allan Kemp Sasha Shafikhani	Nigel Quinn	Teresa Fan Richard Higashi

Monitoring and Data Evaluation:
The monitoring and data gathering required for successful completion of this project will complement the existing compliance monitoring program being undertaken by the cooperating agencies in the SJDS and the CALFED-sponsored "Real-Time San Joaquin River Water Quality Management" project, being undertaken by the SJRMP Water Quality Subcommittee.

Local Support/Coordination with Other Programs:
The proposed project supports a comprehensive plan to establish a real-time monitoring and water quality forecasting system in the San Joaquin Basin including all the major east-side tributaries, the west-side agricultural water districts and the main stem of the San Joaquin River. The project will contribute crucial biological data and new water quality monitoring tools to this effort. The project will support the documentation, validation and accreditation of a selenium removal biotreatment plant located in the Panoche Water District. Using ecophysiological modeling data developed by the UCB group, the treatment system has consistently reduced >80% of the selenium and >90% of the nitrate loading from an agricultural drainage wastestream.

Compatibility with CALFED Objectives:
Selenium has been identified by the SJRMP Executive Council as a water quality stressor of concern in the San Joaquin River. Management of wetland drainage discharges through scheduling of releases to coincide with periods of San Joaquin River assimilative capacity can improve San Joaquin River water quality. No systematic data collection program has been undertaken to date to evaluate the role of microbiota in selenium fate and transport within the SJDS. These datasets are crucial to developing knowledge-based strategies for real-time drainage management. Such a data collection program would also support adaptive management options

that are integral to current SJRMP and CALFED-sponsored initiatives on real-time water quality management in the San Joaquin River and with the Vernalis Adaptive Management Program (VAMP), a multi-agency experiment to improve the San Joaquin River fishery through manipulation of tributary flows and flow release schedules.

Project Title: Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives

IV. PROJECT DESCRIPTION

Background:

Among the water quality issues in the San Francisco Bay-Delta region, selenium (Se) ecotoxicity represents one of the most complex problems. This is because of the extensive bioaccumulation and biogeochemical transformations of Se throughout the aquatic foodchain, which in turn dictate Se impact on the ecosystem. The recognition that the Se foodchain transfer pathway is highly dependent on a given site conditions (e.g. lentic versus lotic) further complicates the issue and necessitates the need for establishing site-specific water quality criteria for Se (see report from EPA's "Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation"). For example, despite the low waterborne Se concentrations (well below the EPA's recommended 5 µg/L limit) observed throughout the Bay-Delta, the Se body burden of an invader species of clam (Asian clam, *Potamocorbula amurensis*) (Brown and Luoma, 1995) and the resident sturgeon species (Kroll and Doroshov, 1991) was found to be above the hazard level. The impact of such a high Se body burden in sturgeon, particularly in reproductive system tissue, is unclear. Chronic Se exposure may negatively impact sturgeon populations since Se is a reproductive stressor, causing tetratogenesis in bird and fish species (e.g. Ohlendorf et al., 1993; Lemly, 1993). Much less is known regarding the impact of the Se pathway on other fish species of the Bay-Delta ecosystem.

A major knowledge gap exists in our understanding of the unusual Se foodchain transfer pathway of the Bay-Delta ecosystem: namely the role of primary production by the microbial community in foodchain transfer from water to top predators. Bacteria and algae comprise the majority of biomass in the Bay-Delta system (see Figure 1), however there has been no systematic data collection effort to determine the effects of these communities on Se fate and transport.

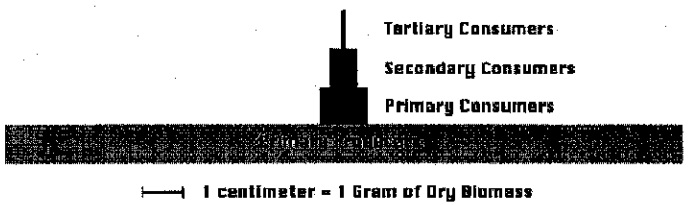

Figure 1

In particular, data relevant to the effects of primary producers on Se biodynamics (e.g. the conversion of selenate into selenoamino acids, elemental selenium, sedimentary selenite, etc.) is severely lacking. Recognition of this knowledge gap represents one consensus opinion in the aforementioned EPA report. Therefore, any change in water quality (resulting from different management practices) of source waters that significantly alters the microbial community, could lead to changes in the Se foodchain transfer pathway and its ecological impact. These effects are well illustrated by the observed changes in

the phytoplankton community (USGS), Asian clam invasion (Carlton et al., 1990), and Se bioaccumulation in Asian clam and sturgeon.

In this proposal, we will investigate how Se biotransformation mechanisms alter microbial biomass foodchain transfer characteristics and therefore, ecotoxic risk. New tools for near real-time monitoring of SJDS microbial communities will be developed. This information is critical to assessing the biological assimilatory capacity of Se in the San Joaquin River, which is in turn needed for managing Se discharge from the San Luis Drain on a real-time basis (Calfed Project D252). In addition, the knowledge gained from this study will help guide the management of the demonstration facility for bacterial Se removal from agricultural drain waters at the Panoche Water District (Firebaugh, CA) (Calfed Project B273). Moreover, these new data will complement the ongoing foodchain studies in fish species of the Delta (Calfed Project B103) and in aquatic birds of the San Joaquin Watershed (Dr. M. Fry, funded by UC Salinity/Drainage program).

Proposed Scope of Work:

The following tasks will be performed during the 2 year duration of the project;

1. Characterize microbial community structure, function and dynamics in a selenium biotreatment facility located in the Panoche Water District.
   - 1.1. Assess microbial community structure and seasonal variation by direct isolation of microbiota and subsequent classification using Biolog and 16S rRNA gene sequencing methods.
   - 1.2. Assess the effects of treatment system operating parameter variations on microbial community structure.
   - 1.3. Assess the ability of representative isolates and communities to assimilate and biotransform selenate and selenite into organic, elemental and volatile selenium species.
     - 1.3.1. Advanced environmental measurement methods including GC/MS, XANES and EXAFS will be used to determine selenium fate and chemical species.
   - 1.4. Assess the effects of treatment system operating parameter variation on microbial community assimilation and biotransformation of selenate and selenite into organic, elemental and volatile selenium species.
   - 1.5. Develop near real-time selenium treatment system microbiota monitoring methods by using Biolog profiling of microbial community metabolic signatures.
     - 1.5.1. Correlate variations in Biolog community level physiological profiles (CLPP) with variations in treatment system operating parameters.
     - 1.5.2. Develop a CLPP database documenting normal, abnormal and recovering microbial community signatures typical of potential variations in treatment system operating modes.

2. Characterize microbial community structure, function and dynamics in Mud Slough and the San Joaquin River.
   - 2.1. Assess microbial community structure and seasonal variation by direct isolation of microbiota and subsequent classification using Biolog and 16S rRNA gene sequencing methods.
   - 2.2. Assess the effects of drainage system operating parameter variations on microbial community structure.
   - 2.3. Assess the ability of representative isolates and communities to assimilate and biotransform selenate and selenite into organic, elemental and volatile selenium species.
     - 2.3.1. Advanced environmental measurement methods including GC/MS, XANES and EXAFS will be used to determine selenium fate and chemical species.
   - 2.4. Assess the effects of drainage system operating parameter variation on microbial community assimilation and biotransformation of selenate and selenite into organic, elemental and volatile selenium species.
   - 2.5. Develop near real-time drainage system microbiota monitoring methods by using Biolog profiling of microbial community metabolic signatures at Mud Slough and San Joaquin sites.
     - 2.5.1. Correlate variations in Biolog community level physiological profiles (CLPP) with seasonal fluctuations in the drainage system environment.
     - 2.5.2. Develop a CLPP database documenting microbial community signatures typical of variations in drainage system operating modes.
     - 2.5.3. Correlate CLPP signature data with real-time monitoring project data to develop selenium adaptive management strategies.
   - 2.6. Assess the ecotoxic risks of bioincorporated selenium species by pure compound and biomass foodchain transfer studies with two resident Bay-Delta species, red swamp crayfish (*Procambarus clarkii*) and mosquito fish (*Gambusia*) receptors.

Location of Project:

The Panoche and Grassland Water Districts are a 50,000 acre area to the north and south of Los Banos on the west side of the San Joaquin Valley, located within Merced County. The project area includes approximately 90 miles of wetland channels and is bounded by the Main Canal and Delta Mendota Canal to the west and the San Luis Drain to the east. Wetland drainage from the Grassland Water District is conveyed to the San Joaquin River through either Mud Slough (north) or Salt Slough.

V. ECOLOGICAL/BIOLOGICAL BENEFITS

Selenium (Se) entering the lower San Joaquin River (SJR) is the primary stressor. Discharge into the SJR of agricultural drainage high in Se is a serious contaminant problem in the lower SJR basin and Bay-Delta. Selenium has caused reproductive failure in sensitive fish species and developmental deformities in waterfowl and shorebirds because of its ability to bioaccumulate to levels that can be toxic to higher trophic organisms. Project benefits include: (1) generation of microbial community structure, function and dynamics data to assess impacts on selenium loading that address deficiencies in the current CALFED-sponsored real-time water quality forecasting project on the mainstem of the SJR; (2) near real-time microbiota monitoring tools for use in the control of a selenium bioremoval demonstration project within the Panoche Water District; (3) near real-time microbiota monitoring tools for use in the current CALFED-sponsored real-time water quality forecasting project on the mainstem of the SJR; (4) an understanding of the ecotoxic risk of bioincorporated selenium to SJR fish and invertebrate species; and (5) the potential for increasing the frequency of meeting SJR water quality objectives.

Spring releases of water from seasonal wetlands are discharged into tributaries of the Lower SJR. These releases, in combination with agricultural drainage that flows through the GWD, contain varying amounts of selenium. Selenium has been identified as a stressor that leads to frequent exceedance of water quality objectives established for the San Joaquin River by state and federal agencies.

Research conducted by Grober et al. (1995) suggests that wetland drainage from the GWD could be scheduled to coincide with peak assimilative capacity in the San Joaquin River to help improve downstream water quality. In addition, increased water supply allocations under the Central Valley Project Improvement Act (CVPIA) have created opportunities to coordinate the release of seasonal wetland drainage with the assimilative capacity of the San Joaquin River. Coordinated releases will help to achieve water quality objectives and improve fish habitat in the main stem of the San Joaquin River and Sacramento - San Joaquin Delta. Improved scheduling of west-side discharges can assist in avoiding critical time periods for fish rearing and remove an important stressor leading to improvements in the San Joaquin salmon fishery. To date, however, no systematic data collection program has been undertaken to elucidate the effects of microbial communities on selenium dynamics and to incorporate these insights into real-time wetland drainage management.

Management of wetland drainage through scheduling of releases to coincide with periods of San Joaquin River assimilative capacity can help to improve San Joaquin River water quality. However, these actions may need to consider the biological impacts of changes to traditional wetland management practices. Peak assimilative capacity typically occurs between the months of January and April. This time period is often times earlier than the traditional wetland draw-down period (March-April). In particular, the response of migratory waterfowl and shorebirds to an early draw-down regime needs to be assessed to determine potential impacts to foraging rates, habitat availability, and species diversity and abundance. It is possible that early, experimental draw-down may make food sources available to wildlife without negatively affecting wetland vegetation community and plant species diversity - hence benefiting both wildlife and the San Joaquin River.

This project should have considerable technology transfer value to other agencies that operate seasonal wetlands and also discharge constituents of concern to the SJR.

The project's microbiota monitoring activities will increase the understanding of factors that affect SJR water quality. This information provided by this secondary benefit can be used to assess the impact of other management practices that attempt to reduce the pollutant load into the lower SJR and Bay-Delta. Species and species groups benefiting from reductions in contaminants entering the Bay-Delta are delta smelt, longfin smelt, splittail, white and green sturgeon, striped bass, resident fish species, marine/estuarine fishes and large invertebrates, Bay-Delta aquatic foodweb organisms, and waterfowl.

Non-ecological CALFED objectives addressed by project include improving SJR and Bay-Delta water quality for agricultural, drinking water, industrial, and recreational beneficial uses. The project will provide data that will facilitate the control and timing of wetland and agricultural drainage to coincide with periods when dilution flow is sufficient to achieve CALFED water quality concentrations.

Linkages:
The described data collection is consistent with the current CALFED-sponsored initiative on real-time water quality management in the San Joaquin River and with the Vernalis Adaptive Management Program (VAMP), a multi-agency experiment to improve the San Joaquin River fishery through manipulation of tributary flows and flow release schedules. Linked projects are :

1. The Coordinated Regional Management Program (CRMP) actions to reduce contaminant loading produced by ephemeral rainfall runoff events in the Panoche Creek Watershed. Selenium generated by Panoche-Silver Creek affects the assimilative capacity of the SJR.
2. Grassland Bypass Project. This project limits monthly selenium loads from the Grassland agricultural water districts and hence affects the assimilative capacity of the SJR.

System Wide Ecosystem Benefits:
The proposed project will provide basic monitoring, decision support tools, and selenium bioremoval system information to allow managers in the SJDS to respond to the long-term challenge of improving water quality while maximizing ecosystem functions and habitat values. Information obtained through this project will be transferable and of significant value to all operators in the SJDS. The successful implementation of this combined monitoring, experimentation, and evaluation program will provide the basis for adaptive management of agricultural drainage throughout the SJDS.

Compatibility with Non-Ecosystem Objectives:
The proposed project is a key element of an expanded CALFED SJR real-time forecasting project. The CALFED San Joaquin River Real-Time Water Quality Management Project uses telemetered stream stage and salinity data and computer models to simulate and forecast water quality conditions along the lower SJR. The decision support tools being used in the existing CALFED project are applicable to the proposed project -the data derived from microbiota monitoring sites will feed into the existing SJRIODAY model to help improve the accuracy of west-side water quality forecasts especially during spring months when seasonal drainage of up to 50,000 acres of

wetlands occurs. At the present time the SJRIODAY forecasting model relies on historic patterns of wetland release in estimating Mud Slough flow and selenium loads.

The monitoring and data gathering required for successful completion of the proposed project will also complement existing compliance monitoring program being undertaken by the cooperating agencies in the GBP and the routine monitoring performed by the CRWQCB and GWD.

VI. TECHNICAL FEASIBILITY AND TIMING

Over the past five years the project team has developed, tested and deployed all of the tools required for the successful completion of the proposed scope of work. In the following sections examples of these efforts and their relevance to CalFed objectives will be described in order to establish the technical feasibility of the proposed workplan.

Microbial Assimilation of Selenium Analysis Tool:

We have demonstrated previously that a well characterized laboratory strain of the common soil and aquatic Gram-positive bacterium, *Bacillus subtilis*, can detoxify soluble Se by aerobic reduction to an insoluble and nontoxic form, elemental selenium (Garbisu et al., 1995; Garbisu et al., 1996; Garbisu et al., 1997). We have developed a chemically defined minimal growth medium and Atomic Absorption Spectroscopy methods to quantify the assimilation of soluble selenium species (selenite and selenate) by pure microbial cultures and microbial communities. *B. subtilis* is able to grow and detoxify soluble Se at concentrations up to 400 ppm. At these high Se concentrations, the primary biotransformed Se species is elemental Se. The Se valence transformation to nontoxic elemental selenium is not affected by a ten-fold excess of nitrate or sulfate - alternate electron acceptors which block Se reduction in anaerobic treatment systems (Garbisu et al., 1995). We conclude that soluble Se is not reduced via dissimilatory electron transport but rather via a novel detoxification system. These results indicate that the soil bacterium *B. subtilis* and related organisms form the basis of a very promising technology for bioremediating selenite. At lower soluble Se concentrations typical of SJDS sites - 100 ppb - *B. subtilis* was able to remove 96% of the Se from the liquid phase (see Figure 2). These results highlight the importance of microbiota in Se bioconcentration. In these experiments the microbiota occupy 1/1000 of the bulk liquid phase volume. Hence, biomass assimilated Se is concentrated 1000X above the level found in the water column.

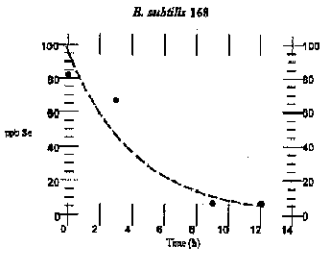

Figure 2 Selenite Removal During Growth of *B. subtilis*

At these environmental Se concentrations, the primary biotransformed Se species are selenoamino acids. A high energy carbon source, such as glucose, supports optimal Se reduction. These results have been used to design and demonstrate a bacterial selenium removal treatment system constructed in the Panoche Water District in collaboration with the UC Algal Research Group (Calfed Project B273).

Several hundred individual bacterial strains have been isolated from the Agatha Canal, San Luis Drain and the Panoche Water District. A majority of these isolates are Gram-negative bacteria that were able to assimilate Se with

kinetics similar to well characterized laboratory strains of *B. subtilis*. At the lowest soluble Se concentration studied - 150 ppb - these isolates were able to remove >98% of Se from the liquid phase (see Figures 3 and 4).

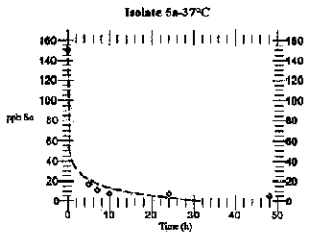

Figure 3

Selenium Removal During Growth of Isolate 5a

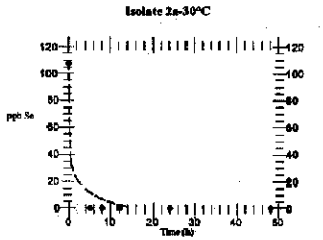

Figure 4

Selenium Removal During Growth of Isolate 2a

It is clear from these data that the microorganisms resident in the SJDS are capable of bioassimilating Se and removing it from bulk liquid phase. On-going studies are directed at taxonomically identifying the Se removing members of the SJDS microbial population (principally Gram-negative groups such as pseudomonads) and assessing the effects of seasonal SJDS and algal/bacterial Se treatment plant operation parameters on the structure, function and dynamics of these microbial ecosystems.

Application of the Microbial Assimilation of Selenium Analysis Tool to CalFed Objectives: The above described methods will be used to assess the selenium removal capabilities of microorganisms and microbial communities isolated from Mud Slough and SJR real-time monitoring sites (Tasks 2.1). These methods will also be used to assess the selenium removal capabilities of microorganisms isolated from the Panoche biotreatment plant (Tasks 1.1). A SJDS culture collection will be established containing representative isolates from each of the monitoring and treatment sites. These organisms will be identified by Biolog and gene sequencing methods (see following sections). Representative isolates will also be used to produce well characterized biomass for Se feeding and foodchain transfer studies (Task 2.6).

Advanced Environmental Measurement Tools for Selenium Speciation:

In collaboration with Roger Prince from EXXON Corporate Research and Ingrid Pickering at the Stanford Synchrotron Research Laboratory (SSRL) we have developed X-ray Absorption Spectroscopy (XAS) techniques to the *in situ* characterization of selenium fate and transformation in aquatic microbial ecosystems. The goal of the project has been to speciate selenium contaminants incorporated into biological sinks to identify selenium valence transformation and bioimmobilization mechanisms in natural environments. There is an urgent need for innovative environmental measurement technologies that are capable of *in situ* condensed phase toxic metal speciation. The XAS techniques pioneered at SSRL have the potential for *in situ* speciation of toxic metals, such as selenium, in sediments and microbial biomass at ppm concentration levels, without physical or chemical manipulation of the sample.

XAS spectra reflect the wavelength-dependent intensity reduction of the incident beam when it passes through a sample. Inner shell electrons of the absorbing atoms, excited by X-ray photons to the continuum and interacting with neighboring atoms, cause a modulation of the absorption coefficient. The fine structure characterizing the spectra, called Extended X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near Edge Structure (XANES) can be used to determine the short range order surrounding specific species and to obtain chemical bonding information. EXAFS refers to the sinusoidal variation of the X-ray absorption coefficient as a function of X-ray photon energy. Oscillations in the post edge region arise from back-scattering of the emitted electron wave by neighboring atoms. The demonstration by Pickering and coworkers (*Environ. Sci. Technol.* **1995**, *29*, 2456) of the ability of XAS to speciate toxic metals, such as Se, *in situ* has created a powerful new tool for the analysis of undisturbed metal species in native environmental samples. Analyses at SSRL of pure culture and microcosm biomass has established that EXAFS is capable of *in situ* speciation of inorganic and organic forms of selenium in these samples. Se removal system microcosms were established and incubated for 48 hours in the presence of 0 - 50 ppm of added soluble Se. The biomass was harvested, frozen and analyzed by EXASF. Table 1 (following page) illustrates the effects of increasing Se concentration on the fractional Se species distribution in the microcosm biomass. The initial soluble Se concentration in the liquid phase was 80 ppb. As Se concentration increased, an increasing fraction of the total biomass Se was in the elemental form. The high fraction of organic Se species observed at the lower Se concentration ranges is consistent with our analysis of the microbial ecology of the treatment system. The predominant microbial populations in the treatment system are Gram-negative bacteria that are known to incorporate selenite into selenoamino acids. At higher and more biologically stressful levels of selenium, the majority of the soluble Se is detoxified to the non-toxic elemental species. Previous studies by our laboratory (Buchanan et al., 1995) have shown that all of the Se in *B. subtilis* biomass exposed to 79 ppm selenite is present as the elemental species.

Table 1

Soluble Se Addition (ppm)	0	0.5	1	10	50
Selenite (% of total)	10.3	7.5	5.9	2.7	0
Selenocyanate (% of total)	5.8	7.3	8.2	8.0	0
Selenomethionine (% of total)	62.3	63.4	63.4	23.1	0
Elemental Selenium (% of total)	21.6	21.8	22.5	66.2	100

Similar XAS Se speciation studies (Table 2) have been conducted with sediments collected by Nigel Quinn from the Agatha Canal. This drainage canal flows from south to north. The south influent stream is high velocity, while the north end of the canal has considerably slower flow and can allow ponding. During the sampling period the north site was also much more productive in terms of microbiota biomass concentration. The data in Table 2 establish that soluble Se species are biotransformed into selenoamino acids by indigenous SJDS microbial communities. These data also provide one of the first demonstrations that SJDS soluble Se is incorporated into biological Se sinks within sediments.

Table 2

Sediment Collection Site	South Site	North Site
Selenate	3.3	3.2
Selenite	54.5	38.5
Selenomethionine	13.2	28.6
Elemental Selenium	29.0	29.7

Application of Advanced Environmental Measurement Tools for Selenium Speciation to CalFed Objectives:

The goals of Tasks 2.3 and 2.4 are to exploit XAS monitoring tools to characterize the biotransformed and bioincorporated Se species that are found in microbial communities isolated from Mud Slough and SJR real-time monitoring sites. The goals of Tasks 1.3 and 1.4 are to exploit XAS monitoring tools to characterize the biotransformed and bioincorporated Se species that are found in microbial communities isolated from the Panoche biotreatment plant. These data are crucial to identifying the mechanisms, sinks and chemical species of Se that are associated with SJDS microbial community assimilative activity. Knowledge of the chemical species abundance and distribution of bioincorporated Se is also required for the design of pure compound and biomass foodchain transfer studies (Task 2.6). These data are crucial to document and validate the mechanisms, sinks and chemical species that are associated with Se removal by the Panoche biotreatment plant (Tasks 1.3 and 1.4).

Biolog, Gene Sequencing and Community Level Physiological Profile (CLPP) Tools for Assessing Microbial Community Structure and Dynamics:

Biolog, Inc. a California Corporation, has developed an unique automated system for fingerprinting, tracking and classifying pure cultures and microbial communities from a variety of environmental samples. Communities of microbes can be directly inoculated into Biolog's 96 well MicroPlate test panels.

Figure 5 Biolog MicroPlate

After incubation a "metabolic fingerprint" characteristic of that community is obtained by scanning the MicroPlate with an optical reader system. The fingerprints have been shown to be both unique and reproducible. These fingerprinting patterns allow the near real-time analysis of ecological systems and the detection of detrimental changes at an early stage. The technology also allows modeling of the affect of seasonal and operational effects on a ecological system. Biolog's test panels incorporate a novel redox chemistry to perform carbon source utilization tests for bacterial identification and fingerprinting. The chemical sensor responds to the process of metabolism (i.e. respiration) rather than to the metabolic by-products (e.g. acid). Thus, Biolog's chemistry is universal, greatly simplifying the testing process and unifying microbial identification under one single chemistry. The components of Biolog's chemistry are prefilled and dried in 96-well microplates containing 95 different carbon sources. The resulting 95-test color/turbidity patterns provide high resolution identification at species and subspecies levels. The identification procedure is simple and fast. Bacteria or environmental samples are inoculated into the MicroPlate. This takes about one minute. After incubation for either 4 hours or overnight the resulting pattern is read either with Biolog's automated MicroStation instrument or by eye. Visual reading is easy because only one color (purple) is involved. Because quantitative interpretation of color reactions is imprecise by eye, the automated MicroStation is required for high resolution studies.

Figure 6 Biolog MicroStation

The MicroStation System allows the user to perform ecological analysis and also identify organisms included in Biolog's database of over 1100 species of microorganisms. We have utilized the Biolog MicroStation for the analysis of ecological samples from sediments, water, and wastewater treatment systems. The MicroStation provides a superior, cost-effective alternative to labor-intensive conventional microbial identification methods such as strips or panels. The 95 carbon-source tests in the Biolog MicroPlate are automatically read and interpreted by the MicroStation instrumentation. Pure culture isolates are then identified in seconds from Biolog's extensive data base of over 1,100 species/groups -- a data base many times larger than strip or panel-based identification systems. Species identifications appear on the computer screen within seconds, along with biotype patterns, a list of closely related species, and other useful statistics. Intelligent software compensates automatically for different color/turbidity intensities, eliminating the subjectivity of visual interpretations. The software allows users to create custom databases for the analysis of unknown species or microbial community fingerprints. Cluster analysis can be performed with graphic output in the form of dendrograms, two-dimensional plots, and three-dimensional plots.

The inability to characterize *in situ* microbial communities rapidly and economically has been a severe barrier to the efficient optimization and control of biological treatment processes and the management of aquatic ecosystems. Successful development of a functionally-based high-throughput methodology for the characterization of microbial communities, the community-level physiological profile (CLPP), provides a critical enabling technology for minimizing the environmental impacts of Se loading. CLPP can provide significant benefits in terms of cost savings, throughput, and sensitivity over existing baseline technologies for microbial treatment system monitoring (e.g., direct microscopic observation, plate counts, biochemical measurements, etc.). CLPP is also useful for microbial community monitoring in association with the the adaptive management of ecosystem performance. The community-level physiological profile (CLPP) was first conceived by Garland and Mills (Garland, J. and A. L. Mills. 1991. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Community-Level Sole-Carbon Source Utilization. Appl. Environ. Microbiol. 57:2351-2359) to distinguish microbial communities from diverse habitats, and along gradients within a given habitat. This assay involves inoculating whole communities from environmental samples into Biolog microbial identification system GN (Gram-negative) microtiter plates and evaluating respiration of ninety-five different sole carbon sources by an automated microtiter plate reader and associated data analysis software. The multivariate dataset is analyzed by principal components analysis (PCA) which extracts major trends in the dataset and allows distinctions between communities that can be correlated with the original variables [sole carbon sources] and perturbations of the ecosystem.

We have demonstrated the power of the CLPP technology is a three year pilot study of the microbial community residing within the a selenium impacted wastewater treatment system. This project had two goals: (i) the development of monitoring tools to assess microbial community adaptation and adjustment to variations in treatment system operating parameters; and (ii) the use of CLPP as a high-throughput screening system to engineer the microbial community metabolically for optimal toxic metal bioremediation. CLPP has proven to be a very sensitive and incisive tool for assessing the integrity and functionality of the treatment system microbial community. CLPP has been used to track a treatment system upset that was caused by an alteration in the food to mass ratio of the wastewater feed stream. This perturbation resulted in significant negative impacts on treatment system nitrification and settling parameters. The three figures in the Appendix plot the CLPP signatures of the Normal Community (Figure 7), the Upset Community (Figure 8), and the Recovering Community (Figure 9). In each of these figures the respiration rate is plotted for a particular test substrate. The surprising result from these studies is that treatment system microbial communities have very simple and diagnostic CLPP signatures. We interpret these results to mean that these communities are highly differentiated metabolic specialists, rather than metabolic generalists which would be expected to exhibit far more complex CLPP signatures than we have observed. The "Normal" CLPP pattern has been stable over three years of treatment system monitoring.

Over the past fifteen years, molecular tools have forever changed studies of microbial evolution and taxonomy by providing more robust phylogenetic frameworks based upon objective genetic criteria. Comparisons of small subunit ribosomal rRNA sequences (16S-like rRNAs) are particularly valuable for phylogenetic inference. The small subunit rRNA database comprises the

largest collection of sequences that share a common ancestry (presently over 1400 microbial entries). These genes are present in all cells where they perform similar functions. Since ribosomal RNA genes do not undergo lateral transfer between organisms, molecular trees inferred from comparisons of their nucleotide sequences accurately depict the historical evolution of their corresponding genomes.

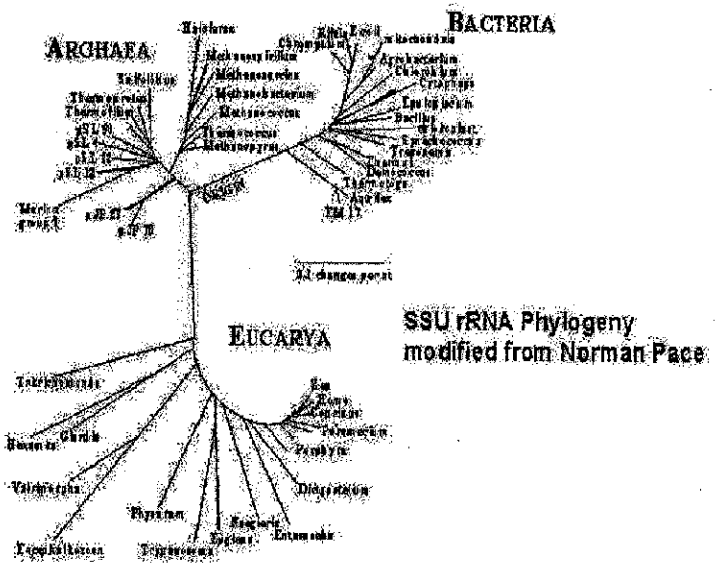

Figure 10 16S rRNA Phylogenetic Tree of Life

Ribosomal RNA coding regions display a mosaic of conservation patterns. Rapidly evolving regions are interspersed among domains that are moderately conserved or nearly invariant in all organisms. This conservation pattern permits rRNA genes to function as "multi-handed chronometers" of evolution. The slowly evolving regions record genetic events that occurred billions of years ago. The most conserved elements presumably reflect functional domains established in the earliest common ancestors to all living systems. The rapidly evolving elements chronicle very recent speciation events. Using comparisons of ribosomal RNAs one can infer evolutionary affinities at the kingdom level or resolve relationships within a genus. Full-length small subunit and large subunit rRNA sequences contain many sites that can vary independently. Identification of microorganisms by 16S rRNA sequence is considered the "gold standard" method for microbial taxonomy. This technology is clearly more time consuming and costly than Biolog identification. We have compared the genus and species assignments produced by Biolog and 16S rRNA phylogeny for a suite of environmental isolates from the Panoche biotreatment plant (see Appendix, Table 4). There is generally good agreement between Biolog and 16S rRNA genus and species assignments. Hence, Biolog will be the initial taxonomic tool used to characterization SJDS microbial communities. A subset of high abundance isolates will be studied by 16S rRNA methods. In cases where Biolog identification is uncertain, 16S rRNA sequencing methods will also be employed to determine the genus and species of the isolate. The accumulation of a SJDS 16S rRNA microbiota sequence database will also be of considerable long-tern importance as "gene chip" identification systems become more affordable and available for the monitoring of environmental ecosystems.

Application of Biolog, 16S rRNA Gene Sequencing and CLPP Tools for Assessing Microbial Community Structure and Dynamics to CalFed Objectives:
Biolog will be the primary tool used for the classification of microorganisms isolated from SJDS monitoring sites at the Mud Slough and SJR monitoring sites(Tasks 2.1 and 2.2). Biolog will also be used to classify isolates from the Panoche biotreatment plant (Task 1.1 and 1.2). Biolog CLPP will be used as a near real-time monitoring tool to fingerprint microbial community signatures at the Mud Slough and SJR monitoring sites (Task 2.5). 16S rRNA gene sequencing methods will be used as required to validate Biolog identification and in cases where Biolog is unable to accurately assign a genus and species. Biolog CLPP will be used as a near real-time monitoring tool to fingerprint microbial community signatures at the Panoche biotreatment plant. CLPP fingerprints will be developed for both sites that characterize normal and abnormal operating regimes. CLPP fingerprinting will be used for stability/recovery analysis of monitoring sites, to determine whether there is a defined recovery path, and to validate the potential of this technology for process monitoring and control.

Samples from SJDS monitoring sites will be subjected to CLPP to produce a multidimensional profile of the mixed aerobic heterotrophic community based on sole carbon source utilization in Biolog GN MicroPlates. Communities will be compared on the basis of average metabolic response (AMR), metabolic diversity, and PCA analysis of the multivariate substrate utilization profile. Collateral data on system operating parameters will also be collected and archived. The response of communities to changes in system operating parameters will be quantitatively evaluated. Metabolic patterns of succession over the course of seasonal variations will be investigated during periods of baseline and subnormal community function. These metadata will provide a comprehensive resource for understanding normal and perturbed system operational regimes.

Selenium Foodchain Transfer Analysis Tool:
Two resident species of the San Joaquin River, San Luis Drain, and Delta, red swamp crayfish (*Procambarus clarkii*) and mosquito fish (*Gambusia*), will be used for the bacterial foodchain transfer experiments. Both species are ubiquitously abundant in the proposed region and serve as direct links from the primary/secondary producers to the top predators including those that are threatened (e.g. Sacramento perch, salmon, hardhead minnow, white-faced ibis, California clapper rail, pied-billed grebe, etc). Teratogenic effects have been documented for mosquito fish in Se-contaminated sites (Lemly, 1993). The field abundance of the two species makes it practical to collect samples from relevant sites, which provides the unusual opportunity to relate results from a laboratory feeding study to field observation. Both species are also polytrophic in terms of their diets, which makes them excellent organisms for examining integrating effects of Se derived from various food sources ranging from detritus, producers, to primary and secondary consumers. Moreover, both species are convenient to rear in the laboratory, and only require several months to become reproductive from larval stage, which will greatly facilitate the feeding studies.

Crayfish will be raised in the UCD flow-through system from the egg to reproductive stage, while mosquito fish will be raised from larval to reproductive stage. For the 1st week of development, larval mosquito fish will be fed brine shrimp nauplii and trained to feed on prepared food as described below. The Se-contaminated food will be prepared from bacterial biomass grown from different Se treatments or pure selenium species, encased in gelatin, and graded into appropriate sizes for feeding from larval to adult stages. The gelatin feeding method has been tested successfully on crayfish and is expected to work for mosquito fish as well. The Se treatments will be adjusted such that the bacterial biomass is respectively dominant in selenomethionine, selenocystine, and selenium element (as determined by XANES and/or GC-MS). If necessary for nutritional balance, casein and/or brine shrimp will be included in the feed. Samples will be taken at larval, pre-, and post-reproductive stages, freeze-dried, and pulverized for Se analysis. When practical, target organs including hepatopacreas/egg mass of crayfish and liver/gonad of mosquito fish will be analyzed separately, which should provide a closer link to ecotoxic risk. Se analysis will include total Se measurements by the fluorescence method (Fan et al., 1998a), free and protein-bound selenoamino acids by GC-MS (Fan et al., 1998a&b) or XANES, and elemental selenium by XANES.

Application of the Selenium Foodchain Transfer Analysis Tool to CalFed Objectives: These studies (Task 2.6) will provide unique information on how various bioconcentrated Se metabolites are assimilated from food sources and in turn metabolized in consumers, particularly into the proteinaceous fraction which is generally recognized as the most ecotoxically relevant pool (see EPA's Peer Consultation report). The comparison of Se bioavailability to higher trophic levels from pure compounds and biomass incorporated species will allow a data-driven assessment of the ecotoxic risk posed by Se incorporated into microbial sinks.

VII. MONITORING AND DATA COLLECTION METHODOLOGY

Microbiota monitoring activities will be coordinated through the San Joaquin River Management Program. Data will be freely exchanged between the participating agencies, providing the modellers making forecasts of water quality on the SJR with accurate and timely information.

Biological/Ecological Objectives

The biological and ecological monitoring and data objectives of the project are as follows:

1. Document the effects of changing drainage discharge patterns on microbiota species and microbial community fingerprints (CLPP).
2. Document the effects of changing Panoche biotreatment plant operating parameters on microbiota species and microbial community fingerprints (CLPP).
3. Measure the impact of these changes in operations and timing on water quality in the SJR. Provide data for model simulations to compare with and without-action scenarios.
4. Support the data environment for an adaptive management approach to optimize wetland habitat and maximize water quality benefits in the SJR.

Monitoring Parameters and Data Collection Approach:
Samples from SJDS monitoring sites will be subjected to Biolog, 16S rRNA and CLPP analysis to produce a multidimensional profile of the SJDS microbiota community. Communities will be compared on the basis of average metabolic response (AMR), metabolic diversity, and PCA analysis of the multivariate substrate utilization profile. Collateral data on system operating parameters will also be collected and archived. The response of communities to changes in system operating parameters will be quantitatively evaluated. Metabolic patterns of succession over the course of seasonal variations will be investigated during periods of baseline and subnormal community function. These metadata will be provided to the CALFED San Joaquin River Real-Time Water Quality Management Project.

Monitoring sites will be chosen to coincide with site used by the CALFED San Joaquin River Real-Time Water Quality Management Project. These same areas will be part of an adaptive management experiment for the duration of the project. Biological data will be entered into a spread-sheet program and analyzed using statistical software such as Biolog, SPSS, SAS or NCSS. Null hypotheses of no net change to habitat quality and function will be tested statistically using a paired t-test analysis. The biological data collected during the project term will be summarized in an annual report and the findings disseminated locally through Water District newsletters and in the scientific literature by submission to a peer-reviewed journal.

Table 3. Monitoring and Data Collection Information

BIOLOGICAL/ECOLOGICAL OBJECTIVES			
Hypothesis/Question to be Evaluated	Monitoring Parameter(s) and Data Collection Approach	Data Evaluation Approach	Comments/Data Priority
1. Characterization of microbial communities within the SJDS can be used to estimate primary producer contributions to selenium flux and transport in the San Joaquin River.	Assessment of microbial community structure and dynamics.	Determination of microbial community genus and species composition. Determination of seasonal variations in microbial community structure. Determination of microbial isolate and community selenium removal kinetics. Coordination with CALFED San Joaquin Real-Time Water Quality Management Study to estimate River assimilative capacity and determine opportunities for agricultural drainage discharge.	High-need data required to assess the role of primary producers in selenium dynamics.
2. Characterization of microbial communities within the SJDS can be used to estimate primary producer contributions to selenium bioassimilation and bioaccumulation within the San Joaquin River.	Assessment of microbial isolate and community bioassimilation and biotransformation of soluble selenium species.	Determination of the kinetics and extent of soluble selenium species assimilation and valence transformation by microbial isolates and communities from the SJDS.	High-need data required to assess the role of primary producers in selenium bioaccumulation and biotransformation.

Hypothesis/Question to be Evaluated	Monitoring Parameter(s) and Data Collection Approach	Data Evaluation Approach	Comments/Data Priority
3. Characterization of microbial communities within the Panoche Biotreatment Plant can be used to document the role of these biota in selenium removal processes from the Panoche Water District.	Assessment of microbial community structure and dynamics.	Determination of microbial community genus and species composition. Determination of seasonal variations in microbial community structure. Determination of microbial isolate and community selenium removal kinetics.	High-need data required to document the role of treatment system microbiota in selenium removal processes.
4. Characterization of microbial communities within the Panoche Biotreatment Plant can be used to document the role of these biota in selenium bioassimilation and bioaccumulation.	Assessment of microbial isolate and community bioassimilation and biotransformation of soluble selenium species.	Determination of the kinetics and extent of soluble selenium species assimilation and valence transformation by microbial isolates and communities from the Panoche Biotreatment Plant.	High-need data required to document the role of treatment system biota in selenium bioaccumulation and biotransformation.
5. Community Level Physiological Profile methods can be used to monitor microbial communities in near real-time.	Biolog monitoring of SJDS and Panoche Biotreatment Plant microbial communities.	CLPP signature databases analysis of normal and abnormal system operational fingerprints.	Near real-time monitoring microbiota database for use in adaptive management of selenium flux.

Hypothesis/Question to be Evaluated	Monitoring Parameter(s) and Data Collection Approach	Data Evaluation Approach	Comments/Data Priority
6. Characterization of foodchain transfer of microbiota incorporated selenium can be used to estimate primary producer contributions to selenium ecotoxic risk of within the San Joaquin River.	Assessment of foodchain transfer of microbial isolate and community bioaccumulated selenium species to swamp crayfish and mosquito fish.	Determination of the kinetics and extent of selenium species accumulation and valence transformation by higher trophic level receptors indigenous to the SJDS.	High-need data required to assess the biomagnification and ecotoxic risk of primary producer incorporated selenium to fish and invertebrates in the SJDS.

VIII. LOCAL INVOLVEMENT

Two existing multi-agency programs are already in place to use the information generated by this study data in planning and executing remediation efforts and assisting in evaluating the effect of those efforts. The first program, with the USEPA as lead agency, is that of the Coordinated Resource Management Program (CRMP) for the Panoche/Silver Creek watershed. The second is the Grassland Bypass Project, a multi-agency program to enforce selenium load limits from agricultural water districts within the 100,000 acre Grassland watershed. Letter of support from the Grasslands Area Farmers and the Panoche Water District, as well as a letter of notification to the Merced County Board of Supervisors are included on the following three pages.

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JOSEPH S. SUMMERS
JOSEPH C. MCGAHAN
ROGER L. REYNOLDS
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Note new area code →

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April 13, 1999

COPY

Professor Terrence Leighton
Department Of Cell Biology
401 Barker Hall
UC Berkeley
Berkeley, CA 94720

SUBJECT: Grassland Area Farmers Support for CALFED Grant Proposals

Dear Professor Leighton:

The Grassland Area Farmers have a long history of supporting innovative drainage reduction strategies on the west side of the San Joaquin Valley. As the proponent of the Grassland Bypass Project, the Grassland Area Farmers have invested millions of dollars in the past 3 years to improve monitoring and increase control over subsurface tile drainage leaving the area. Significant reductions in selenium loads contained in these discharges have been necessary to meet the strict selenium load limits imposed by the Project.

The CALFED proposal entitled "Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives" is of great interest to the Grassland Area Farmers. The Grassland Area Farmers long-term plan includes movement toward a goal of real-time management of selenium discharge to the San Joaquin River. As the Grassland Area Farmers progress in their ability to manage selenium drainage this goal becomes more achievable. The development of seasonal, site-specific standards for selenium in the San Joaquin River will benefit the Grassland Area Farmers, providing greater flexibility of operation and at the same time be protective of the ecosystem. We hope the above-mentioned project helps to make progress towards this goal.

Sincerely,

Joseph C. McGahan
Drainage Coordinator for the Grassland Area Farmers

JCM/p

PANOCHE WATER DISTRICT

52027 WEST ALTHEA, FIREBAUGH, CA 93622 • TELEPHONE (209) 364-6136 • FAX (209) 364-6122

April 12, 1999

Professor Terrence Leighton
Department of Cell Biology
401 Barker Hall
UC-Berkeley
Berkeley, CA 94720

Subject: Panoche Water District Support for CALFED Grant Proposals

Dear Professor Leighton:

The Panoche Water District has a long history of supporting innovative drainage reduction strategies on the west-side of the San Joaquin Valley. As a participant in the Grassland Bypass Project the water district has invested millions of dollars in the past 3 years to improve monitoring and increase control over subsurface tile drainage leaving the water district. Significant reductions in selenium loads contained in these discharges have been necessary to meet the strict selenium load limits imposed by the Project.

The CALFED proposal entitled "Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives" is of great interest to the District. The District's long-term plan includes movement toward a goal of real-time management of selenium discharge to the San Joaquin River. As the District progresses in its ability to manage selenium drainage this goal becomes more achievable. The development of seasonal, site-specific standards for selenium in the San Joaquin River will benefit the Grassland Area farmers, providing greater flexibility of operation and at the same time be protective of the ecosystem. We hope the above-mentioned project helps to make progress towards this goal.

Sincerely,

Dennis Falaschi
General Manager

LETTER OF NOTIFICATION

I-018530

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EARTH SCIENCES DIVISION
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March 30, 1999.

Ms. Lydia Beiswanger, Chief Deputy
Merced County Board of Supervisors
2222 M Street
Merced, CA 95340.

Dear Ms. Beiswanger:

This letter is to inform you of our intent to submit a proposal to the CALFED Bay-Delta Program entitled Microbial sensors for selenium hazard assessment and development of site-specific selenium objectives". It has been recognized by scientists involved in selenium research that the 5 ppb concentration objective is insensitive to the spatial and temporal dynamics of the ecosystem - hence the objective may over-restrictive in some locations at certain times and not restrictive enough at other times and a other locations. The objective of this project is to derive more sensitive and accurate biosensors for selenium hazard assessment.

The long term goal of agricultural water districts involved in the Grassland Bypass Project is to develop a real-time forecasting system for selenium loading to the San Joaquin River. As the District progresses in its ability to manage selenium drainage this goal becomes more achievable. The development of seasonal, site-specific standards for selenium in the San Joaquin River will benefit the Grassland Area farmers, providing greater flexibility of operation and at the same time be protective of the ecosystem.

We believe that successful completion of this study will be of great benefit to landowners and water district personnel in the Grassland watershed of Merced County.

Sincerely,

Nigel W.T. Quinn

Geological Scientist

IX. COSTS AND SCHEDULE TO IMPLEMENT PROPOSED PROJECT

The proposed project will have a two year duration with the initiation of microbiota monitoring and pure compound foodchain studies occurring during year 1, the continuation of microbial monitoring and biomass foodchain studies during year 2, and the field coordination with the SJRMP Water Quality subcommittee in both years 1 and 2. Procedures for the isolation, characterization and classification of microbiota, procedures for obtaining microbiota CLPP, and advanced environmental measurement methods for the speciation of Se in biomass, will be established and validated during the first 6 months of the project and will be implemented during the project's two year term.

The work schedule is shown in the table below. Two progress reports and one final project report will be prepared summarizing the objectives accomplished during the year and results from activities in the SJDS. Demonstrations and workshops will be conducted to disseminate results form the project and to introduce potential users to the Biolog CLPP microbiota monitoring technology.

PROJECT MONTH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
REPORTS											x	x											x	x
TASK 1.1		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
TASK 1.2										x	x	x	x	x	x	x	x	x	x	x	x			
TASK 1.3						x	x	x	x	x	x	x	x	x	x	x	x	x						
TASK 1.4												x	x	x	x	x	x	x	x	x	x			
TASK 1.5						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
TASK 2.1		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
TASK 2.2										x	x	x	x	x	x	x	x	x	x	x	x			
TASK 2.3						x	x	x	x	x	x	x	x	x	x	x	x	x						
TASK 2.4												x	x	x	x	x	x	x	x	x	x			
TASK 2.5						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
TASK 2.6			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			

The following pages contain summary budgets and individual institutional budgets for the two year project period. Two budget formats are included: one assuming that the State of California 10% overhead rate applies to the project and another assuming that the Federal 50.1% overhead rate applies to the project. A separate budget is also included for Tasks 1 and 2.

OMB Approval No. 0348-0044

BUDGET INFORMATION -- Non-Construction Programs

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. Task 1		$	$	$ 289,905	$	$
2. Task 2				356,740		
3.						
4.						
5. TOTALS		$	$	$ 646,645	$	$

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY (1)	(2)	(3)	(4)	TOTAL (5)
a. Personnel	$	$	$ 288,946	$	$
b. Fringe Benefits			62,806		
c. Travel			20,000		
d. Equipment			15,000		
e. Supplies			52,000		
f. Contractual					
g. Construction					
h. Other					
i. Total Direct Charges (Sum of 6a - 6h)			438,752		
j. Indirect Charges			207,893		
k. TOTALS (Sum of 6i and 6j)	$	$	$ 646,645	$	$
7. Program Income	$	$	$	$	$

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ITEMIZED BUDGET - UCB

Categories	Year One	Year Two	Total Project
a. Personnel			
PI, T. Leighton	0	0	0
Staff Research Associate	30,200	31,725	61,925
Graduate Student	17,850	18,206	36,056
Lab Assistant	12,120	12,600	24,720
TOTAL PERSONNEL	60,170	62,531	122,701
b. Fringe Benefits			
Normal	15,400	16,415	31,815
TOTAL FRINGE BENEFITS	15,400	16,415	31,815
c. Travel			
Scientific Presentation & Field	5,000	6,000	11,000
TOTAL TRAVEL	5,000	6,000	11,000
d. Equipment			
Equipment	0	0	0
TOTAL EQUIPMENT	0	0	0
e. Supplies and Recharges			
Supplies	14,000	16,000	30,000
Recharges	0	0	0
TOTAL SUPPLIES	14,000	16,000	30,000
f. Contracts	0	0	0
g. Construction	0	0	0
h. Other	0	0	0
i. TOTAL DIRECT COSTS	94,570	100,946	195,516
j. INDIRECT COSTS (less fees)	8,920	9,505	18,425
TOTAL INDIRECT COSTS (10%)	8,920	9,505	18,425
k. TOTAL PROJECT COSTS	103,490	110,451	213,941
l. TOTAL REQUESTED	103,490	110,451	213,941
* Graduate student fees (included w/ benefits)	5,370	5,895	11,265

ITEMIZED BUDGET - UCD

Categories	Year One	Year Two	Total Project
a. Personnel			
PI, R.M. Higashi	5,446	5,718	11,164
PI, T. W-M. Fan	5,732	6,018	11,750
Postdoctoral Scientist	30,380	34,178	64,558
Other	0	0	0
TOTAL PERSONNEL	41,558	45,914	87,472
b. Fringe Benefits			
Normal	9,973	11,018	20,991
TOTAL FRINGE BENEFITS	9,973	11,018	20,991
c. Travel			
Scientific Presentation & Field	1,500	2,000	3,500
TOTAL TRAVEL	1,500	2,000	3,500
d. Equipment			
(see details)	12,000	0	12,000
TOTAL EQUIPMENT	12,000	0	12,000
e. Supplies and Recharges			
Supplies	10,000	12,000	22,000
Instrument Recharges	0	0	0
TOTAL SUPPLIES	10,000	12,000	22,000
f. Contracts	0	0	0
g. Construction	0	0	0
h. Other	0	0	0
i. TOTAL DIRECT COSTS	75,031	70,932	145,963
j. INDIRECT COSTS (less equipment)	6,303	7,093	13,396
TOTAL INDIRECT COSTS (10%)	6,303	7,093	13,396
k. TOTAL PROJECT COSTS	81,334	78,025	159,359
l. TOTAL REQUESTED	81,334	78,025	159,359

ITEMIZED BUDGET - LBNL

Categories	Year One	Year Two	Total Project
a. Personnel			
PI, Nigel Quinn	38,773	40,000	78,773
Other	0	0	0
TOTAL PERSONNEL	38,773	40,000	78,773
b. Fringe Benefits			
Normal	5,000	5,000	10,000
TOTAL FRINGE BENEFITS	5,000	5,000	10,000
c. Travel			
Scientific Presentation & Field	2,500	3,000	5,500
TOTAL TRAVEL	2,500	3,000	5,500
d. Equipment			
Computer & Computer Supplies	1,500	1,500	3,000
TOTAL EQUIPMENT	1,500	1,500	3,000
e. Supplies and Recharges			
Supplies	0	0	0
Recharges	0	0	0
TOTAL SUPPLIES	0	0	0
f. Contracts	0	0	0
g. Construction	0	0	0
h. Other	0	0	0
i. TOTAL DIRECT COSTS	47,773	49,500	97,273
j. INDIRECT COSTS (less equipment)	4,627	4,800	9,427
TOTAL INDIRECT COSTS (10%)	4,627	4,800	9,427
k. TOTAL PROJECT COSTS	52,400	54,300	106,700
l. TOTAL REQUESTED	52,400	54,300	106,700

TOTAL BUDGET

Categories	Year One	Year Two	Total Project
a. Personnel TOTAL PERSONNEL	$140,501	$148,445	$288,946
b. Fringe Benefits TOTAL FRINGE BENEFITS	$30,373	$32,433	$62,806
c. Travel TOTAL TRAVEL	$9,000	$11,000	$20,000
d. Equipment TOTAL EQUIPMENT	$13,500	$1,500	$15,000
e. Supplies and Recharges TOTAL SUPPLIES	$24,000	$28,000	$52,000
f. Contracts	$0	$0	$0
g. Construction	$0	$0	$0
h. Other	$0	$0	$0
i. TOTAL DIRECT COSTS	$217,374	$221,378	$438,752
j. INDIRECT COSTS (less equipment/fees) TOTAL INDIRECT COSTS (10%)	$19,850	$21,398	$41,248
k. TOTAL PROJECT COSTS	$237,224	$242,776	$480,000
l. TOTAL REQUESTED	$237,224	$242,776	$480,000

ITEMIZED BUDGET - UCB

Categories	Year One	Year Two	Total Project
a. Personnel			
PI, T. Leighton	0	0	0
Staff Research Associate	30,200	31,725	61,925
Graduate Student	17,850	18,206	36,056
Lab Assistant	12,120	12,600	24,720
TOTAL PERSONNEL	60,170	62,531	122,701
b. Fringe Benefits			
Normal	15,400	16,415	31,815
TOTAL FRINGE BENEFITS	15,400	16,415	31,815
c. Travel			
Scientific Presentation & Field	5,000	6,000	11,000
TOTAL TRAVEL	5,000	6,000	11,000
d. Equipment			
Equipment	0	0	0
TOTAL EQUIPMENT	0	0	0
e. Supplies and Recharges			
Supplies	14,000	16,000	30,000
Recharges	0	0	0
TOTAL SUPPLIES	14,000	16,000	30,000
f. Contracts	0	0	0
g. Construction	0	0	0
h. Other	0	0	0
i. TOTAL DIRECT COSTS	94,570	100,946	195,516
j. INDIRECT COSTS (less fees)	44,957	47,906	92,863
TOTAL INDIRECT COSTS (50.4%)	44,957	47,906	92,863
k. TOTAL PROJECT COSTS	139,527	148,852	288,379
l. TOTAL REQUESTED	139,527	148,852	288,379
* Graduate student fees (Benefits)	5,370	5,895	11,265

ITEMIZED BUDGET - UCD

Categories	Year One	Year Two	Total Project
a. Personnel			
PI, R.M. Higashi	5,446	5,718	11,164
PI, T. W-M. Fan	5,732	6,018	11,750
Postdoctoral Scientist	30,380	34,178	64,558
Other	0	0	0
TOTAL PERSONNEL	41,558	45,914	87,472
b. Fringe Benefits			
Normal	9,973	11,018	20,991
TOTAL FRINGE BENEFITS	9,973	11,018	20,991
c. Travel			
Scientific Presentation & Field	1,500	2,000	3,500
TOTAL TRAVEL	1,500	2,000	3,500
d. Equipment			
(see details)	12,000	0	12,000
TOTAL EQUIPMENT	12,000	0	12,000
e. Supplies and Recharges			
Supplies	10,000	12,000	22,000
Instrument Recharges	0	0	0
TOTAL SUPPLIES	10,000	12,000	22,000
f. Contracts	0	0	0
g. Construction	0	0	0
h. Other	0	0	0
i. TOTAL DIRECT COSTS	75,031	70,932	145,963
j. INDIRECT COSTS (less equipment)	31,768	35,750	67,517
TOTAL INDIRECT COSTS (50.4%)	31,768	35,750	67,517
k. TOTAL PROJECT COSTS	106,799	106,682	213,480
l. TOTAL REQUESTED	106,799	106,682	213,480

ITEMIZED BUDGET - LBNL

Categories	Year One	Year Two	Total Project
a. Personnel			
PI, Nigel Quinn	38,773	40,000	78,773
Other	0	0	0
TOTAL PERSONNEL	38,773	40,000	78,773
b. Fringe Benefits			
Normal	5,000	5,000	10,000
TOTAL FRINGE BENEFITS	5,000	5,000	10,000
c. Travel			
Scientific Presentation & Field	2,500	3,000	5,500
TOTAL TRAVEL	2,500	3,000	5,500
d. Equipment			
Computer & Computer Supplies	1,500	1,500	3,000
TOTAL EQUIPMENT	1,500	1,500	3,000
e. Supplies and Recharges			
Supplies	0	0	0
Recharges	0	0	0
TOTAL SUPPLIES	0	0	0
f. Contracts	0	0	0
g. Construction	0	0	0
h. Other	0	0	0
i. TOTAL DIRECT COSTS	47,773	49,500	97,273
j. INDIRECT COSTS (less equipment)	23,322	24,192	47,514
TOTAL INDIRECT COSTS (50.4%)	23,322	24,192	47,514
k. TOTAL PROJECT COSTS	71,095	73,692	144,787
l. TOTAL REQUESTED	71,095	73,692	144,787

TOTAL BUDGET

Categories	Year One	Year Two	Total Project
a. Personnel TOTAL PERSONNEL	$140,501	$148,445	$288,946
b. Fringe Benefits TOTAL FRINGE BENEFITS	$30,373	$32,433	$62,806
c. Travel TOTAL TRAVEL	$9,000	$11,000	$20,000
d. Equipment TOTAL EQUIPMENT	$13,500	$1,500	$15,000
e. Supplies and Recharges TOTAL SUPPLIES	$24,000	$28,000	$52,000
f. Contracts	$0	$0	$0
g. Construction	$0	$0	$0
h. Other	$0	$0	$0
i. TOTAL DIRECT COSTS	$217,374	$221,378	$438,752
j. INDIRECT COSTS (less equipment/fees) TOTAL INDIRECT COSTS (50.4%)	$100,046	$107,847	$207,893
k. TOTAL PROJECT COSTS	$317,420	$329,225	$646,645
l. TOTAL REQUESTED	$317,420	$329,225	$646,645

Summary Budget(By Task)

Tasks	Direct Labor Hours	Direct Salary & Benefits	Service Contracts	Material & Acq. Costs	Misc. & Other Direct Costs	Total Direct Costs	Indirect Costs (Fed. Rate @ 50.4% MTDC)	Total Costs (Fed. Rate)	Indirect Costs (State Rate @ 10% MTDC)	Total Costs (State Rate)
Task 1	-	150,604	-	13,350	32,400	196,354	93,552	289,906	18,561	215,999
Task 2	-	201,148	-	1,650	39,600	242,398	114,342	356,740	22,687	264,001
Project Mgt. Task	-	-	-	-	-	-	12,163	-	-	
Grand Totals	-	$ 351,752	$ -	$ 15,000	$ 72,000	$ 438,752	$ 220,057	$ 646,646	$ 41,248	$ 480,000

Task1 Budget

TASK 1	Direct Labor Hours	Direct Salary & Benefits	Service Contracts	Material & Acq. Costs	Misc. & Other Direct Costs	Total Direct Costs	Indirect Costs (Fed. Rate @ 50.4% MTDC)	Total Costs (Fed. Rate)	Indirect Costs (State Rate @ 10% MTDC)	Total Costs (State Rate)
UCB	-	61,805	-	-	18,450	80,255	41,788	122,043	8,291	96,273
UCD	-	48,805	-	12,000	11,475	72,280	30,383	102,663	6,028	71,711
LBNL	-	39,994	-	1,350	2,475	43,819	21,381	65,200	4,242	48,015
Project Mgt. Task	-	-	-	-	-	-	-	-	-	
Grand Totals	-	**$ 150,604**	**$ -**	**$ 13,350**	**$ 32,400**	**$ 196,354**	**$ 93,552**	**$ 289,906**	**$ 18,561**	**$ 215,999**

Task2 Budget

TASK 2	Direct Labor Hours	Direct Salary & Benefits	Service Contracts	Material & Acq. Costs	Misc. & Other Direct Costs	Total Direct Costs	Indirect Costs (Fed. Rate @ 50.4% MTDC)	Total Costs (Fed. Rate)	Indirect Costs (State Rate @ 10% MTDC)	Total Costs (State Rate)
UCB	-	92,711	-	-	22,550	115,261	51,075	166,336	10,134	117,668
UCD	-	59,658	-	-	14,025	73,683	37,134	110,817	7,368	87,648
LBNL	-	48,779	-	1,650	3,025	53,454	26,133	79,587	5,185	58,685
Project Mgt. Task	-	-	-	-	-	-	-	-	-	
Grand Totals	-	**$ 201,148**	**$ -**	**$ 1,650**	**$ 39,600**	**$ 242,398**	**$ 114,342**	**$ 356,740**	**$ 22,687**	**$ 264,001**

X. COST SHARING

The Bureau of Reclamation, CalFed (Calfed Project B273), Exxon Corporation, SSRL and the US Army Corps of Engineers have provided previous funding which supported collection of the preliminary data cited in this proposal. A portion of the CalFed and USACE funds will be used for cost sharing during the two year period of the project. The project will have access to Atomic Absorption Spectroscopy and Biolog instrumentation in the UCB BEST facilities. Professor Leighton is the PI of a DOE grant from the SSRL for XAS speciation of selenium in environmental samples by XANES and EXAFS. SLAC beam time will be used for Selenium speciation of CalFed microbiota samples. A portion of Professor Leighton's salary is provided by the University of California.

The Panoche Water District is providing in-kind contributions to the proposed project.

XI. APPLICANT QUALIFICATIONS

The team members include UCB, LBNL, and UCD personnel all of whom have worked in the SJDS for the past five to ten years. The UCB group has specialized in developing tools for the analysis of microbial community structure, function and dynamics in selenium impacted environments. The UCB group has also developed X-ray absorption spectroscopy tools for the *in situ* determination of selenium species and distribution in microbial biomass. The LBNL group has specialized in SJDS selenium fate and transport experiments. LBNL has also developed fate and transport models to support real-time adaptive management of selenium loading. The UCD group has specialized in developing tools for the analysis of algal community structure, function and dynamics in selenium impacted environments. The UCD group has developed GC/MS tools for the determination of selenium species and distribution in algal biomass. The UCD group is recognized for their ability to assess selenium foodchain transfer characteristics and ecotoxic risk.

Professor Terrance Leighton (Microbiology and Biochemistry, UCB)

Profesor Leighton has been a faculty member at UC Berkeley for the past twenty five years. He directs the UCB Bioremediation, Education, Science and Technology Center. Professor Leighton is an expert in microbial biology, microbial ecology, the molecular mechanisms which regulate hazardous metal detoxification and biosorption in bacteria, and the microbial ecophysiology of wastewater treatment systems and damaged environments.

ADMINISTRATIVE POSITIONS:

Director UCB Bioremediation Education Science and Technology Center
Founding Member - European Science Foundation Phytoremediation Scientific Network
UCB Biocomputing Coordinator
CoDirector UCB - CalEPA Bioremediation Validation and Certification Laboratory
Director UCB Advanced Undergraduate Biotechnology Research Program
Founding UCB Member - Science Education Academy of the Bay Area (SEABA)
UC Systemwide Biotechnology and Bioengineering Grant Review Committee

Dr. Nigel Quinn (Geological Scientist, ESD, Lawrence Berkeley National Laboratory)

Nigel Quinn received a BSc (Hons) in irrigation engineering and hydrology from the Cranfield Institute of Technology in England and spent the early part of his career as an irrigation engineer for Tate and Lyle Inc. designing and troubleshooting irrigation systems in England and in Africa. He left England for Iowa in 1978 where he taught agricultural water management, rural water supply engineering and surveying courses for three years, earning an MS in Agricultural and Civil Engineering and conducting research in soil erosion under crop canopy. In 1981 he took a position at Cornell University where he worked on various projects ranging from earthworm vermicomposting, pesticide model development and water supply and sanitation policy in developing countries, co-taught classes in surveying and computer programming and earned a PhD

in civil and environmental engineering in 1987. He then joined the San Joaquin Valley Drainage Program, retaining a faculty affiliation with Cornell, and took responsibility for development of groundwater and drainage models to support the Drainage Program's planning effort. With the sunset of the Drainage Program he has continued his work with the US Bureau of Reclamation dividing his time between monitoring efforts in support of the Grasslands Bypass project, development of real-time forecasting tools for the San Joaquin River and selenium fate and transport research projects. He has been affiliated with Lawrence Berkeley National Laboratory for the past 6 years. Nigel is the author of over 50 publications and reports on various aspects of water resources and drainage engineering.

Dr. Teresa Fan (Associate Research Professor, UCD)

Dr. Teresa W-M. Fan is faculty member in the Department of Land, Air and Water Resources, University of California, Davis. Her research interest has been in the broad area of environmental biochemistry ranging from plant stress biochemistry and Se biogeochemistry in relation to *in situ* bioremediation, to mechanisms of aquatic ecotoxicity of agricultural and industrial discharges. Along CalFed's interest, she has been working on salinity and toxic metals stress on the Asian clam, *Potamocorbula amurensis*, in the Delta/San Pablo Bay, as well as the tradeoffs between algal phytoremediation and ecotoxic risk of selenium in San Joaquin Valley's evaporation ponds. She has served on the 9-member EPA Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation (March 1998) which concluded that selenium organic forms and foodchain biochemistry - not total Se - should be the target of ecotoxic investigations and bioremediation goal. Most recently, she was one of the authors of the Central Valley Drainage Implementation Program's comprehensive report on Discharge to the San Joaquin River.

Dr. Richard Higashi (Assistant Research Professor, UCD)

Dr. Richard M. Higashi is a faculty member in the Crocker Nuclear Laboratory, University of California, Davis. He has worked in broad areas of environmental chemistry, ranging from toxicity identification in complex effluents such as pulpmill and oil production discharges, to DOE waste contamination remediation, to agricultural water, soil, and sediment problems of the Central Valley and San Francisco Bay/Delta, as well as air pollution (PM10 and ozone) research in the Central Valley and Sierra Nevada Range. The chemistry of humics and other organic matter plays a central role in all of these research areas, and he is currently engaged in organic matter chemistry investigations in relation to selenium ecotoxic remediation in evaporation ponds of the SJV.

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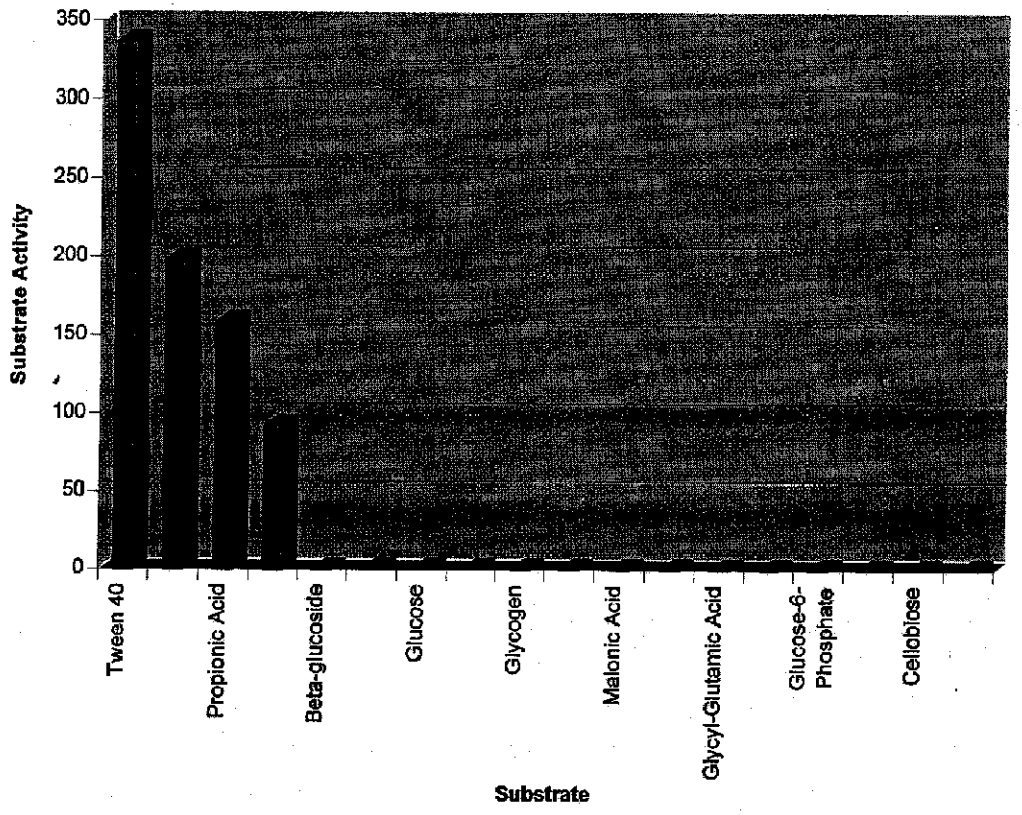
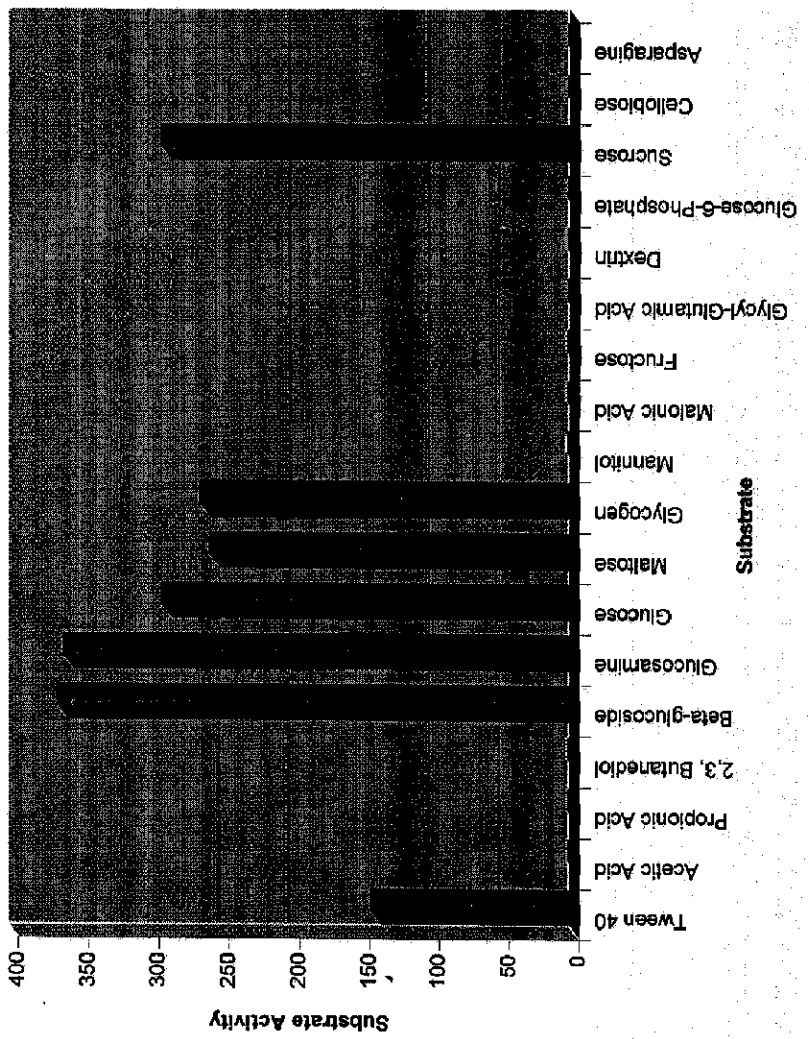
APPENDICES

Appendix Figure 7
Normal BIOX Microbial Community Breathprint



I-018550

Appendix Figure 8
Abnormal BIOX Microbial Community Breathprint 6/28/95



Appendix Figure 9
Recovering BIOX Microbial Community Breathprint 8/18/95

I-018552

Appendix Table 4: 16S rRNA and Biolog Identification of South Agatha Isolates

Strain	16S ID (primer:926,1492r)	Sim.	16S ID (primer: 27f,519r)	Sim.	Biolog Identification	Sim.
432	*Aeromonas sp2*	0.981	*Aeromonas media*	0.949	*Aeromonas media like DNA group 5A*	0.758
433	*Bacillus licheniformis*	1.00	*Bacillus licheniformis*	1.00	*Bacillus licheniformis*	0.789
434	*Bacillus licheniformis*	0.94	*Bacillus licheniformis*	0.94	*Bacillus licheniformis*	0.897
435	*Bacillus licheniformis*	0.973	*Bacillus licheniformis*	0.973	*Bacillus licheniformis*	0.786
437	*Pseudomonas flavescens str. b62*	0.902			*Pseudomonas viridilivida*	0.655
438a	*Brevibacterium acetylicum*	0.83	*Brevibacterium acetylicum*	0.83	*Lactococcus lactis ss hordniae*	0.787
438b	*Pseudomonas mendocina*	0.836	*Pseudomonas mendocina*	0.946	*Pseudomonas viridilivida*	0.600
439	*Bacillus licheniformis*	1.00	*Bacillus licheniformis*	1.00	*Bacillus licheniformis*	0.936
440	*Bacillus species*	0.78	*Bacillus licheniformis*	0.813	*Bacillus megaterium*	0.530
442	*Pseudomonas flavescens*	0.971	*Pseudomonas mendocino*	0.957	*Pseudomonas viridilivida*	0.819
443	*Pseudomonas flavescens*	0.962	*Pseudomonas mendocino*	0.958	*Pseudomonas viridilivida*	0.849
444	*Aeromonas jandace*	0.902	*Pseudomonas mendocina*	0.958	*Aeromonas media like DNA group 5A*	0.715
445	*Bacillus licheniformis*	0.972	*Bacillus licheniformis*	1.00	*Bacillus licheniformis*	0.92
447a	*Aeromonas salmonicida subsp. achromogenes*	0.900	*Aeromonas media like-DNA group 5a*	0.971	*Aeromonas media like DNA group 5A*	0.829
449	*Pseudomonas stutz14*	0.838			*Pseudomonas viridilivida*	0.782